



THE ROLE OF ADENO-ASSOCIATED VIRUSES

Interview with Professor David Schaffer

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Dr. David Schaffer is a Professor of Chemical and Biomolecular Engineering, Bioengineering, and Neuroscience at the University of California, Berkeley. Professor Schaffer is interested in stem cell bioengineering, gene delivery systems, molecular virology, and their applications to biomedical problems. In this interview, we talk about the role of adeno-associated viruses in gene therapy and discuss its molecular basis and directed evolution approaches.



Professor David Schaffer
[Source: The Schaffer Lab]

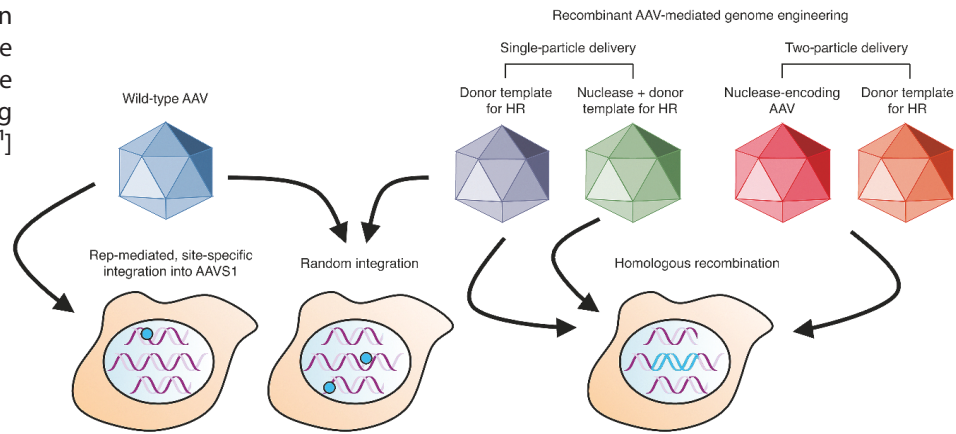
BSJ: How did you first get involved in research in Chemical and Biomolecular Engineering?

DS: Well, I come from a family with a medical background both on the basic sciences side and the clinical side. I was always interested in problems related to human health. I like molecules and I like thinking about problems quantitatively. So, if you put that all together - math, molecules, application towards healthcare - at the time, it was Chemical and Biomolecular Engineering. These days I think that this research takes place in both the CBE department as well as the Bioengineering department and reflecting that I have an appointment in both.

BSJ: What has inspired your interest specifically in gene therapy?

DS: Well, I began to work in gene therapy during graduate school (that was a number of years ago, I probably shouldn't tell you as it is going to date me), so I have been working in the field for over 20 years. At that time, there was a lot of excitement: people were talking about sequencing the human genome, the Human Genome Project was getting underway. The genes that cause haemophilia B, haemophilia A, cystic fibrosis, muscular dystrophy, and Huntington's disease were getting cloned and sequenced and it brought up the idea that DNA could be used as a medicine to be able to treat diseases. I thought that this would be revolutionary and got really excited because up until that point many of

Scheme of AAV integration into the human genome
[Source: Genome Engineering Using Adeno-associated Virus ¹]



these diseases were simply untreatable. The big challenge that emerged in the field was that in many situations you could identify down to the base pair the sequence of DNA that you needed to deliver to be able to treat the disease; however, delivering a sufficient amount of DNA to enough cells was a problem. That's where we really have set our sites in the past couple of decades.

BSJ: Some of your publications focus on the use of adeno-associated viruses (AAVs) for gene therapy. What has made an AAV such a highly promising gene delivery vector?

DS: Several things. One is that it is harmless. All of us have been previously infected with a natural version of this virus and never even noticed because it doesn't cause human disease. It is a safe virus, one that is somewhat stealthy as far as the immune system goes - it doesn't cause major inflammatory responses. A second thing is that it has some level of gene delivery efficiency to a number of different cell types. Compared to other viruses that lack the ability to infect, for example, a muscle cell or a neuron, this virus is more efficient.

BSJ: What is the mechanism by which AAVs infect the cells and integrate into the genome?

DS: You can think of an AAV as a ball of protein surrounding a string of DNA. The surface of that ball

"We are going to create better gene delivery vehicles and ... translate this towards clinical development"

of protein is what interacts with the body. For example, let's say you would like an AAV to go to the liver and deliver a therapeutic piece of DNA. Let's say you want to deliver a gene-encoding factor 9 (a blood clotting protein that's missing in patients that have haemophilia B). If you inject AAV into the bloodstream, it will penetrate deep into the liver tissue, recognise the surface of an hepatocyte (a liver cell), bind it, get internalized, traffic to the nucleus, and then uncoat. The virus naturally evolved over millions of years and accumulated those properties over time. We are taking advantage of natural evolution - we ironically view this virus as gift of nature - trying to improve the efficiency with which it carries DNA inside the cells. You asked the final question: how does DNA make it into the nucleus? Recombinant AAV is a non-integrating virus so it lacks the ability to integrate itself or insert itself into the genome. As long as the cell is not a dividing, the virus will persist for many years. We like this fact because integration could potentially damage the genome.

BSJ: What's the role of the helper virus when AAV is used?

DS: You need a helper virus in order to get AAV to replicate. If you are trying to manufacture the virus in culture to be able to produce it for therapeutic use you need it to make many copies of the virus. AAV is called an "adeno-associated" virus because it was originally isolated as a contaminant with an adenoviral stock and it requires the presence of an adenovirus to be able to replicate.

BSJ: So what makes the efficiency of AAVs slightly higher than that of other vectors?

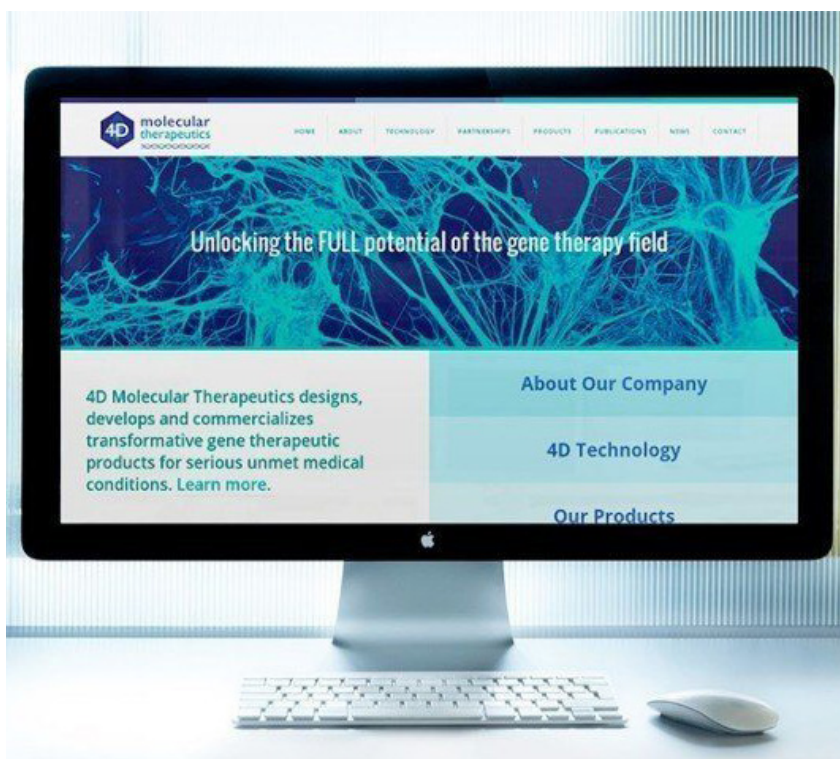
DS: Well, it's evolution. It is a respiratory virus, so it evolved primarily within the lung. We don't fully understand the evolutionary history of the virus but we're given a particle that has the ability to make it into cells

at a reasonable level. You obviously wouldn't want to use something like wild type HIV, for example, as a delivery vehicle. HIV is highly specific to infecting T-cells and macrophages and maybe a couple of other cell types. The evolutionary forces that drove HIV into being the virus it is today are very specific for those cells and we couldn't use HIV for infecting liver or neurons, but AAV was given to us by nature as something that had a reasonable level of infectivity on a broad range of cells.

BSJ: How can directed evolution be employed to engineer vectors with enhanced properties? When are directed evolution approaches² more useful than rational design?

DS: The answer to the second question has so far been "always." This is our contribution to the field; we invented the concept of applying directed evolution to make better gene delivery vehicles, to make better AAVs. The idea is that nature created AAV over tens of millions of years for its own purposes - it's a relatively successful respiratory virus. If you showed AAV a neuron or photoreceptor or muscle stem cell (any number of cells that are ther-

apeutically important target cells), most of the time the virus would say: "What the heck is this? I have not been ever evolutionarily rewarded in my lifetime for the ability to deliver DNA to this cell." In one sense, evolution has given us this virus that's a good starting point but we need to improve on it and make it much more successful for our applications because nature never evolved it for our convenience to use it as a medicine. But evolution in general is a very powerful engine to create novel and useful biological function. So, what we've been doing is accelerated artificial evolution in the laboratory. Evolution has two steps: to create a very large and diverse gene pool and then to select the fittest. We create enormous gene pools on the order of 100 million viruses and we select the best ones for their ability to infect a neuron or a photoreceptor or a muscle stem cell, or whatever the target cell is for the particular disease we want to treat. Directed evolution, as we've developed it, is a very effective and powerful approach to create highly optimized versions of AAV for gene delivery to any cell or tissue target in the body. People had been doing rational design previously, but the challenge or the problem there is that, like I mentioned, this ball of protein has been endowed by nature with the



Co-founded by Professor Schaffer, the Berkeley company 4D Molecular Therapeutics develops and commercializes transformative gene therapeutic products
[Source: <http://www.4dmolecularterapeutics>]

ability to interact with the bloodstream, endothelium, tissue, cell surface, endosome, cytosol, and nucleus. It's a really complicated delivery pathway and if this ball of protein is not very good at getting into that neuron, that's all we know – it doesn't make it into the neuron. We don't know which of these steps along the way is responsible, mechanistically, for the poor delivery efficiency. To enable rational design to work, we need to know lots of molecular mechanisms of that full pathway. Rational design requires a lot of information to be able to design something that's actually going to work. Evolution functions in the near absence of mechanistic information, so it's much faster, more efficient and we can always after the fact reverse engineer the final product and understand mechanistically why it worked and therefore what was the nature of the problem to begin with. But it's always nice to be approaching mechanism while having the solution in hand.

BSJ: What are some of the obstacles associated with using AAV technology? For example, how has your group addressed the body's immune response to the virus or penetration of dense tissues?

DS: Going back to that list of potential barriers again: interaction with the bloodstream with components of the blood system, interaction with the tissue, getting deep into the tissue, being able to target delivery to the desired cell type and then very efficiently being able to infect that target cell. Each one of those steps has been found in different situations to be a rate-limiting step. In our very first publication, we had dealt with that very first step, which is essentially the fact that all of us have been infected with this virus naturally. We have high concentrations of antibodies, which are our body's initial natural defence systems against viruses. These pre-existing antibodies will neutralize natural versions of the virus because our body doesn't know the difference between a natural virus and a therapeutic virus and it's going to reject both. As a result, in most clinical trial today, patients who have antibodies against the natural version of AAV used in that trial are excluded from the trial. We have been evolving and engineering new versions of the virus that are resistant to the majority of antibodies in the human population. We're going to be able to enroll a significantly higher fraction of people in the population in the trial and ultimately a higher fraction of potential patients will benefit from the therapy. Another example in the past two weeks: we've had papers coming out dealing

with the infection step – that last step where the virus needs to make it very efficiently into the target cell. In one paper, we created a version that's about 300-fold better on infecting the airway epithelium and lung and, in another paper, a version that's 100-fold better in infecting neurons.

BSJ: How can AAV-mediated gene therapy be used to treat neurological disorders such as Parkinson's disease and ALS?

DS: Initially, where I think the field has been focused and should be focused in the past 5-10 years has been on rare monogenic diseases where you can point at the gene and the mutation within the gene that's responsible for the disease. And then it becomes a straightforward hypothesis: If this gene is broken and if I deliver enough of the replacement gene, I should fix the issue. That's where the field is focused primarily right now in haemophilia B, haemophilia A, retinitis pigmentosa, Leber congenital amaurosis (LCA), muscular dystrophy... All of these are situations in which a gene is broken and you need to supply a replacement gene. If these begin the work, in other words, if our vectors get good enough, then we could take on riskier disease targets. Only then we could start going after Parkinson's disease or Alzheimer's or congestive heart failure or type II diabetes. We feel that now we should focus on these rare monogenic diseases where we know exactly where the problem lies and then build up momentum to take on tougher disease targets.

BSJ: How has the discovery of CRISPR Cas 9 impacted research on AAVs?

DS: CRISPR Cas 9 is an incredibly enabling capability. I will give you a couple of examples. In situations where a gene is broken (it is a recessive disorder) and the replacement gene is small enough to fit inside the AAV then we probably don't need genome editing, like in haemophilia B and LCA. In situations where a gene is broken and has gained a function (an autosomal dominant disease) like Huntington's your job is to knock out a gene that has acquired, due to a mutation, a pathological function and is causing the disease. CRISPR Cas 9 can then go in and edit the genome to knock out that disease-causing gene. A third category is situations where it is a recessive disease but the replacement gene is too big to fit inside an AAV. Then you can potentially use Cas 9 and homologous recombination to fix the genome. Cas 9 is incredibly enabling for genetic therapies, but it, like other cargos,

needs a delivery vehicle. I think that it is synergistic: if we end up creating the optimized vehicles and here is this terrific cargo then a new generation of molecular medicines can be created.

BSJ: You have briefly mentioned this before, but how effective are AAVs in clinical trials?

DS: There is actually an approved gene therapy in Europe that's based upon AAV. In the United States, there is a treatment for a blinding disorder called Leber congenital amaurosis type II (for which a company has completed a phase 3 clinical trial). There is going to be a BLA (Biologics Licensing Application) to the FDA, which is seeking approval to market a drug. That BLA will be filed next year. Hopefully, based on very positive results in the phase 3 trial, that's going to lead to the very first approval of a gene therapy in the United States. Earlier, there have been several clinical trials with positive results for haemophilia B and some diseases within the nervous system, like spinal muscular atrophy. These are situations where the natural versions of the virus are just good enough to start getting this efficacy and, in addition, in some cases, it is not a complete rescue, it is a partial rescue, so we feel that if we could create delivery vehicles that are 10 or a 100 fold better, then we could start going after tougher disease targets.

BSJ: What future steps do you plan to take in your research?

DS: Well, several things. The university is an incredible incubator for innovative technologies. We are going to continue to create better vehicles, better ways of making delivery vehicles and better cargos within our lab here. At the same time, we feel that we should also be translating this towards clinical development. The goal is to get the technology into as many patients' trials and product as we possibly can. Clinical development takes place within the private sector, so several years ago I co-founded a company at Berkeley called 4D Molecular Therapeutics (<http://www.4dmoleculartherapeutics.com/>) and we are taking this technology and getting it into clinical development both within the company as well as in partnership with other companies like Pfizer and Roche Pharma.

BSJ: Thank you very much for your time!

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